

- 26 -

CLAIMS

1. A method of culture of mycobacteria, comprising culturing said mycobacteria, in batch fermenter culture or continuous culture, with agitation and in the presence of at least 0.1% (v/v) detergent.
2. A method according to Claim 1, comprising culturing the mycobacteria at a temperature of 35°C +/- 10°C.
3. A method according to Claim 1 or 2, comprising maintaining the pH at 6.9 +/- 0.9.
4. A method according to any of Claims 1 to 3, comprising culturing the mycobacteria with an initial dissolved oxygen concentration of at least 1% (v/v) air saturation.
5. A method according to any of Claims 1 to 4, for culture of mycobacteria selected from *M. tuberculosis*, *M. bovis* and *M. vaccae*.
6. A method according to any of Claims 1 to 5 for batch culture of mycobacteria, wherein detergent is present at from 0.1 to 1.0 % (v/v).
7. A method according to Claim 6, wherein detergent is present at about 0.2 % (v/v).
8. A method according to any of Claims 1 to 5 for continuous culture of mycobacteria.

AMENDED SHEET
IPEA/EP

- 27 -

9. A method according to Claim 8, wherein detergent is present at at least 0.15 % (v/v).
10. A method according to Claim 8 or 9, wherein the culture is carried out continuously with a dilution rate of at least 0.02 h^{-1} .
11. A method according to Claim 10, wherein the culture is carried out continuously with a dilution rate of at least 0.025 h^{-1} .
12. A method according to Claim 8 or 9, comprising growing said mycobacteria in continuous culture, at a temperature of $35^{\circ}\text{C} \pm 10^{\circ}\text{C}$, at a dissolved oxygen tension of at least 1 percent, at a pH of 6.9 ± 0.9 , at a dilution rate of at least 0.02 h^{-1} .
13. A growth medium for culture of mycobacteria, comprising:-
a carbon source;
a mitogen;
trace elements comprising at least Mg, K, P and S;
a nitrogen source; and
at least 0.1% (v/v) detergent.
14. A growth medium according to Claim 13, wherein the carbon source is selected from glucose, glycerol and an amino acid.
15. A growth medium according to Claim 13 or 14, wherein the mitogen is asparagine.
16. A growth medium according to any of Claims 13 to 15, comprising trace

- 28 -

elements selected from Ca, Mg, Zn, Co, Cu, Mn, Fe, K, and mixtures thereof.

17. A growth medium according to any of Claims 13 to 16, wherein the nitrogen source is selected from an amino acid and an ammonium salt.

5

18. A growth medium according to Claim 17, comprising an amino acid component selected from alanine, arginine, asparagine, aspartic acid, glutamic acid, glycine, isoleucine, leucine, phenylalanine, serine and mixtures thereof.

10

19. A growth medium according to any of Claims 13 to 18, further comprising a vitamin/co-factor component selected from inositol, thiamine, calcium pantothenate, co-enzyme A, nicotinamide, biotin, DL-thioctic acid, and mixtures thereof.

15

20. A medium according to any of Claims 13 to 19, further comprising one or more components selected from sodium hydroxide, glutathione, glycerol, haemin, sodium pyruvate and α -ketoglutarate.

20

21. A method according to any of Claims 1-12, comprising culturing said mycobacteria in the presence of a growth medium according to any of Claims 13 to 20.

25

22. A method of culture of mycobacteria substantially as hereinbefore described with reference to the examples.

23. A growth medium substantially as hereinbefore described with reference to the examples.

- 29 -

24. A method of culture of a mycobacteriophage, comprising culture of mycobacteria according to any of Claims 1-12, 21 or 22, and contacting said mycobacteria with a mycobacteriophage.
- 5 25. A method according to Claim 24, comprising challenging the mycobacteria with an agent for promoting and/or assisting mycobacteriophage adsorption on the mycobacteria.
- 10 26. A method according to Claim 24, wherein challenge occurs prior to or substantially at the same time as contacting the mycobacteria with the mycobacteriophage.
- 15 27. A method according to any of Claims 24-26, comprising reducing or minimising exposure of the phage to detergent present in the mycobacteria culture medium.
28. A method according to Claim 27, comprising allowing a phage infection to be established, and increasing the detergent concentration to at least 0.1% (v/v) detergent.